# GLIAL FIBRILLARY ACIDIC PROTEIN IN PRIMARY ASTROGLIAL CELL CULTURES DERIVED FROM NEWBORN RAT BRAIN

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## 1. Introduction

In a recent study on brain specific proteins in a primary culture of astroglial cells derived from newborn rat brain, the glial fibrillary acidic protein (GFA) was found to be enriched more than forty times in the astroglial culture compared to whole brain [1]. A series of neuronal membrane proteins (D1, D2, D3 and synaptin) were absent, and only trace amounts of the neuronal cytoplasmic protein 14-3-2 were present in the culture at day 21, thus supporting the astroglial nature of this primary culture. The present communication reports the concentration of GFA in the primary culture at various times of cultivation and correlates the findings to immunoperoxydase staining of the cultivated cells using antiserum to GFA.

## 2. Materials and methods

Cultures were obtained from the cerebral hemispheres of newborn Wistar rats (approx 24 h after birth) as described by Booher and Sensenbrenner [2]. The meninges were removed and the hemispheres of six rats gently squeezed through a nylon sieve (80 μm pore size) into 20 ml modified Eagle's medium [3] supplemented with 20% (v/v) fetal calf serum. The cells were grown in Falcon flasks (30 ml vol). Suspension, 1 ml, and 3 ml medium were introduced into each flask which was kept at 37°C in atmospheric air mixed with 5% (v/v) $CO_2$. The medium was changed after 2 days, removing all unattached cells, and subsequently changed three times per week. Cells for GFA measurements were obtained at days 8, 15, 19 and 28; cells used for histochemistry were obtained at days 8, 15, 21 and 28.

For GFA determination the nutrient medium was removed and the cultures washed twice in 10 ml serum-free medium (pH 7.3, 37°C), loosened by a rubber policeman and centrifuged 1000 × *g* for 10 min. The supernatant was removed leaving only a few μl above the cell pellet, which was subsequently stored at −70°C.

Rat brain tissue for GFA determination was obtained at days 7, 14, 21 and 28 after birth. Animals of either sex were decapitated, the forebrain removed

and frozen as described above. The pelleted cells were suspended in an extraction medium consisting of 2–4% w/v Berol-EMU 043 (MoDo-Kemi, Sweden), 20 mM Tris–barbital buffer, pH 8.6, 15 mM $NaN_3$, 100 KIE/ml aprotinin (Trasylol®, Bayer, FRG). Rat forebrains were homogenized in a glass-teflon Potter-Elvehjem homogenizer with a clearance of 0.1 mm, for 2 min in the above-mentioned extraction medium. The detergent to protein ratio was 2–3 in all samples. Total protein concentration was determined in the suspensions according to Lowry et al. [4] using bovine serum albumin as a standard. To all samples 1:10 amount of a 2% sodium dodecyl sulphate solution was added before total protein determination. A monospecific rabbit antiserum was produced against the GFA protein purified as described [5]. GFA was quantified by rocket immunoelectrophoresis [6]. Equipment for immunoelectrophoresis was purchased from Dansk Laboratorieudstyr, Denmark. Agarose was purchased from Litex, Denmark. A Tris–barbital buffer, 20 mM, pH 8.6 was employed in the gels and buffer vessels. Triton X-100 0.2% (Packard, USA) was incorporated in the gels. Immunohistochemical staining: The cultures were washed in phosphate buffered saline (PBS) and immediately fixed for 30 min at 4°C in 1% formaldehyde, freshly made from paraformaldehyde. They were then sequentially incubated in 30 min steps in the following reagents: (1) rabbit antiserum against GFA (diluted 1/100, 1/200, 1/500, 1/1000); (2) peroxidase labelled swine

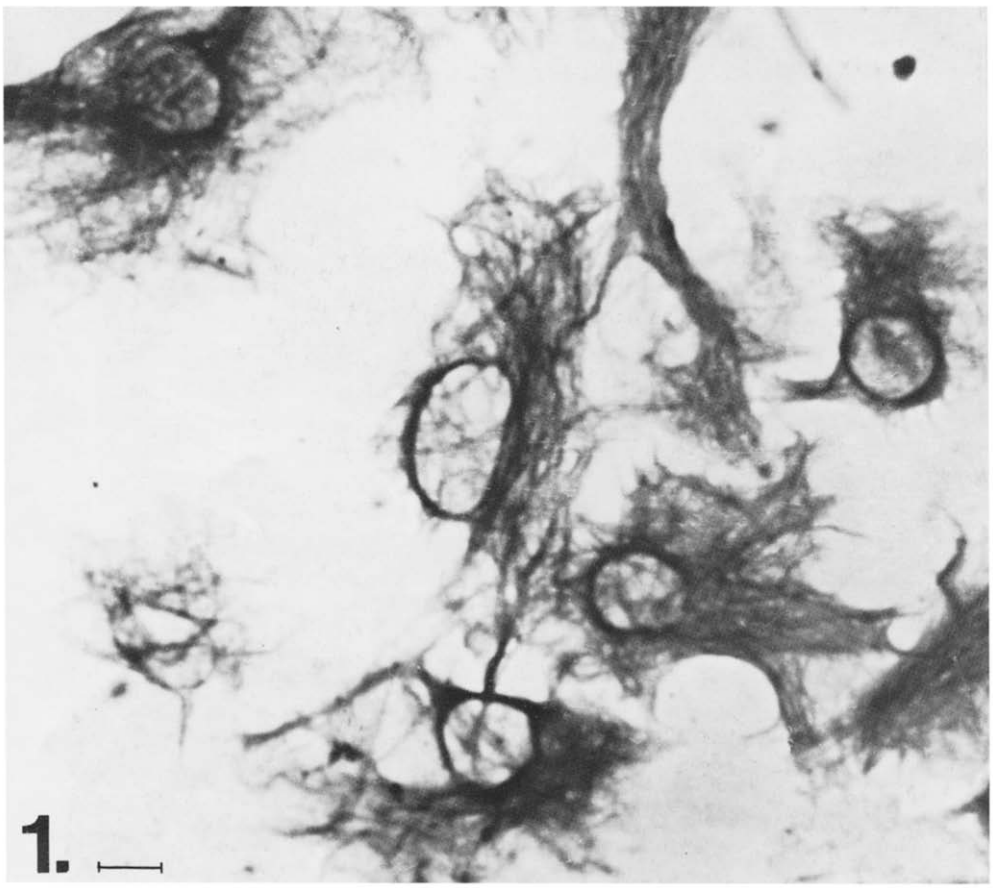


Fig.1. Immunohistochemical reaction for GFA on an eight day old glial culture. Note the network of GFA-positive filaments in the cells. The bar represents 10 μm.

anti-rabbit IgG (diluted 1/20). All the dilutions were performed in PBS. Each step was followed by washing in PBS for 3 X 5 min. By a 5–15 min reaction with a mixture containing 0.05% 3,3′-diaminobenzidine tetrahydrochloride and 0.005% hydrogen peroxide in 0.05 M Tris–HCl buffer (pH 7.6), the peroxidase bound to the cells formed the brown insoluble reaction product. After a 15 min wash in distilled water the sections were treated with 2% $OsO_4$ in water for 5 min, washed in tap water and mounted in glycerol-gelatin.

Details concerning control of specificity of antiserum and of immunohistochemical staining have previously been published [7].

## 3. Results

In the eight day old cultures most of the cells were flat polygonal cells from which few short processes in some cases extended. Few of the cells were bipolar or multipolar cells with long extending processes. All the cells in these cultures were stained by the GFA immunohistochemical procedure, fig.1. The peroxidase reaction product was confined to a cytoplasmatic filamentous network, which was dense in the perinuclear areas.

In the 28 day old cultures the majority of the cells were polygonal cells, but some multipolar cells were also present, especially concentrated in a few selected areas. These multipolar cells had a perikaryon from which 2–8 slender processes emerged. These cells had an appearance like classical astrocytes. The 28 day old cultures often consisted of 2–3 layers of cells. By the immunohistochemical procedure, only the superficial layers were stained, fig.2. In the polygonal cells a filamentous network was stained as in the young cultures, but the filaments tended to be collected in thick bundles radiating out from the nucleus. The multipolar astrocyte like cells were

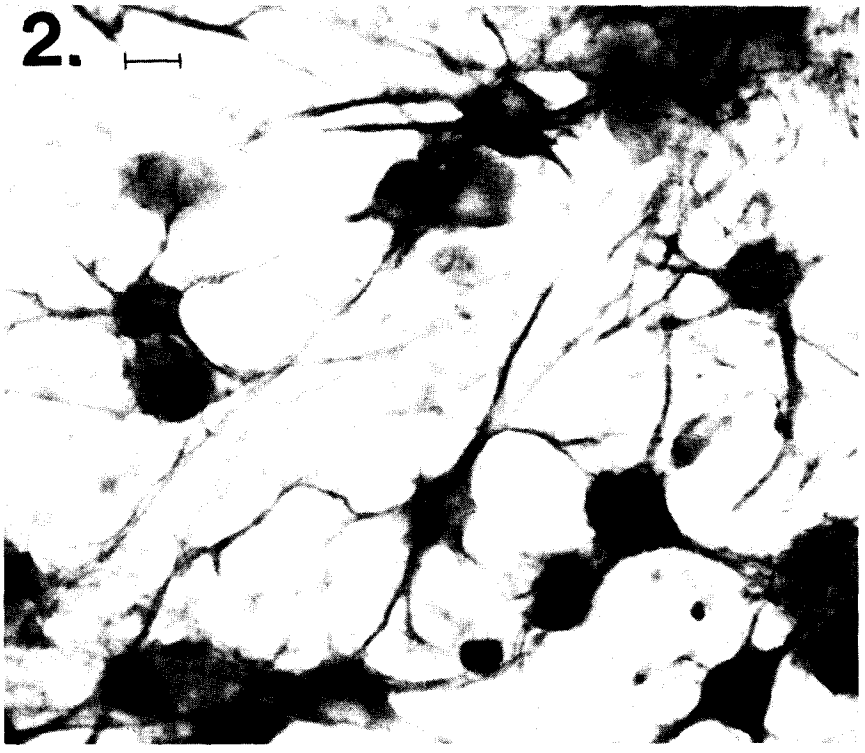


Fig.2. Immunohistochemical reaction for GFA on a 28 day old glial culture. Many cells at this age possess several long processes, which are stained by the immunohistochemical technique together with the cell bodies. The bar represents 10 μm.

Table 1
GFA (a.u.) in primary rat astroglial cell cultures at different ages and in rat forebrain at corresponding ages

| Age days | 7 | 14 | 21 | 28 |
|---|---|---|---|---|
| Forebrain | 1.2 ± 0.16 | 1.7 ± 0.13 | 1.2 ± 0.09 | 1.0 ± 0.15 |
| *n* | 8 | 8 | 8 | 7 |
| Age days | 8 | 15 | 19 | 28 |
| Culture | 4.6 ± 0.73 | 8.5 ± 0.95 | 6.0 ± 1.10 | 15.3 ± 2.07 |
| *n* | 3 | 3 | 4 | 4 |

*n*, number of determinations

The results are given as mean ± SEM

homogeneously stained in both the perikaryon and the processes, fig.2; control staining, fig.3.

The 15 and 21 day old cultures consisted of polygonal and some multipolar cells. The immunohistochemical staining was as described above.

The amount of GFA, extractable by non-ionic detergent, present in the cells at various times of cultivation and in forebrain at corresponding ages, is shown in table 1. The concentration of GFA was expressed as a relative specific concentration with respect to the protein content in the suspensions, setting the specific concentration of non-ionic detergent extractable GFA in normal rat forebrain at age 28 days to 1.0 arbitrary unit (a.u.).

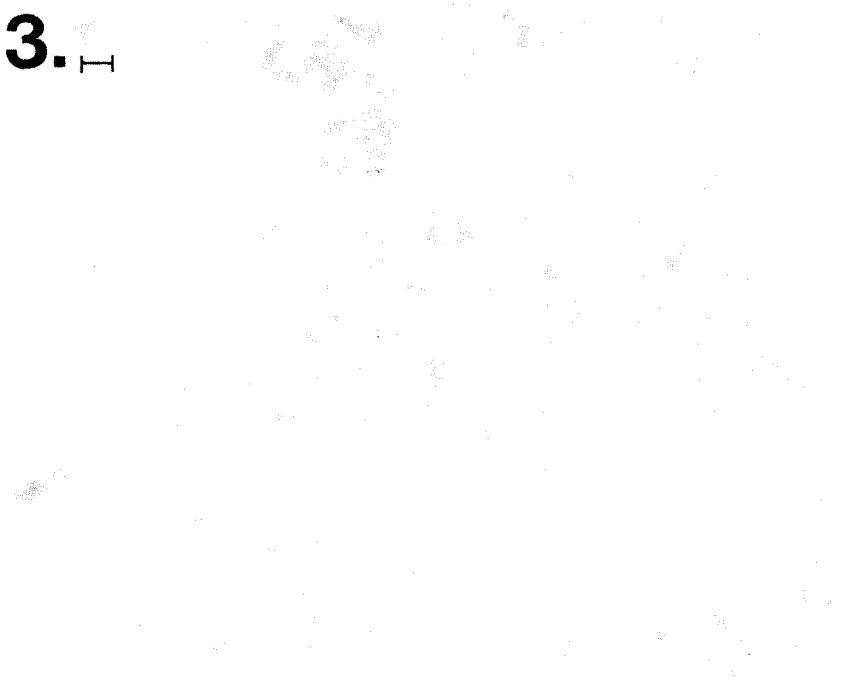


Fig.3. Control picture from a 28 day old glial culture. Rabbit preimmune serum has been used instead of the specific anti-GFA immune serum. The bar represents 10 μm.

## 4. Discussion

By the immunohistochemical staining for GFA, the eight day old cultures showed a fibrillar pattern in the cytoplasm in the polygonal cells. This corresponds well with earlier descriptions of cells stained by the immunohistochemical procedures for GFA [7,8]. The reason why a fibrillar pattern in most cells from the older cultures was not observed may be explained by the high concentration of filaments in these cells, together with the thickness of the cells. In the cultures GFA increased by age, although there seemed to be a slight statistically insignificant decrease from day 14 to day 21. After 28 days of cultivation the cultures contained a 15-times higher concentration of GFA than forebrain at the corresponding age. This enrichment was smaller than that reported [1], but in good accordance with results obtained in a later study (Sensenbrenner and Bock, unpublished).

The concentration course of GFA in whole rat forebrain during ontogeny, with a maximum at day 14 (170% of adult concentration), is in accordance with a previous study on GFA in normal mouse brain during ontogeny, where a peak in concentration was found at day 12 (195% of adult concentration) [9]. It is noteworthy that the peak corresponds to the outburst of astroglial differentiation at the time of myelination.

## Acknowledgements

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